

*** 3511070004 ***

NO. 3511070004

Ready Biodegradation Test of XXXXXXXXXX
(CO₂ Evolution Test)

Test Report No.: 3511070004

Study Director: Haokun Jin

Final Report date: /

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2. Copies of the test report without the official seal of the laboratory are invalid.
3. The test report is invalid without the signatures of Study director, QA Management and Facility management.
4. The test report is invalid if it is altered.
5. It is forbidden to copy the test report partially without the written approval of the laboratory.
6. The conclusion of the consignment test is only valid for the provided sample.
7. In case of discrepancies between Chinese version and English version, the Chinese version shall prevail.

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Summary

The biodegradation test of [REDACTED] was conducted in activated sludge in an aerobic aqueous medium.

[REDACTED] as added to the culture medium at a level equating to 15.2 mg TOC/L. The accumulated percentage biodegradation of the test substance in activated sludge after 2, 4, 6, 8, 10, 12, 14, 18, 22, 26 and 29 days were 1.63%, 3.56%, 5.09%, 6.00%, 7.36%, 7.41%, 7.48%, 7.60%, 7.95%, 8.24% and 8.36%, respectively. Therefore, the accumulated percentage biodegradation rate of the test substance was 8.36% in 29-day.

The accumulated percentage biodegradation of the reference substance (aniline) in activated sludge after 2, 4, 6, 8, 10, 12, 14, 18, 22, 26 and 29 days were 2.09%, 22.31%, 52.67%, 61.15%, 67.84%, 73.66%, 76.57%, 79.64%, 81.28%, 83.03% and 83.19%, respectively.

[REDACTED] was not concluded to be easily (readily) biodegradable in this test condition.

1、 Study Title

Ready Biodegradability of [REDACTED] CO₂ Evolution Test .

2、 Test objective

This study was conducted to determine the biodegradability of the test substance as the nominal sole source of organic carbon in activated sludge in an aerobic aqueous medium. The present study was performed in compliance with the OECD's Guidelines for the Testing of Chemicals No. 301B " CO₂ EVOLUTION TEST" (Adopted: 17th July 1992).

3、 Test method

OECD's Guidelines for the Testing of Chemicals No. 301B " CO₂ EVOLUTION TEST" (Adopted: 17th July 1992).

4、 Study number

3511070004

5、 Experimental date

Starting date : 2011/08/10

Completion date : 2011/09/08

6、 Test facility

Test facility: Shanghai Research Institute of Chemical Industry Testing Centre

Address: No. 345 East Yunling Road, Shanghai

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7、 Name and address of the sponsor

Sponsor: [REDACTED]

Contact: /

Address: [REDACTED]

Tel: /

Fax: /

8、Description of test item

Information supplied by the sponsor:

Name	[REDACTED]
Chemical Name	[REDACTED]
Molecular Formula	[REDACTED]
Batch No	not provided
Appearance	white crystalline powder
Odour	not provided
Solubility in water	(gin 100ml solvent @ 25℃) <0.2
Melting point	183℃
Vapour pressure	not provided
Absorption	not provided
Flammability(solid, gaseous)	not provided
Flash point	not provided
Ignition temperature	not provided
Self-ignition temperature	not provided
Oxidizing properties	not provided
Explosion	not provided
Log Pow	not provided
pH	not provided
purity	99.15%

Conditions of storage not provided

Stability Stable

9、Name and address of test facility management and study personnel

Test facility management: Gang Liu

Study director: Haokun Jin

Study personnel: Yaogao Shu, Fangli Zhou

Address: No. 345 East Yunling Road, Shanghai

10、Description of test methods and materials

10.1 Inoculum from activated sludge

A fresh sample of activated sludge was collected from the aeration tank of Shanghai Sitang Sewage Treatment Center. This plant is a main sewage plant in Shanghai for domestic waste treatment. Some of the sample was shaken for 24 hours, and the coarse particles were removed by filtration through a fine sieve (20 mesh). The sludge was suspended in the mineral nutrient medium to yield a concentration of 3gSS/L–5g SS/L determined with the dry weight method, and aerated until required thereafter.

10.2 Preparation of mineral nutrient medium

The stock solutions were prepared as the following using analytical grade reagents:

(A) 8.50g potassium dihydrogen orthophosphate (KH_2PO_4), 21.75g dipotassium hydrogen orthophosphate (K_2HPO_4), 33.40g disodium hydrogen orthophosphate dihydrate ($\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$), and 0.5g ammonium chloride (NH_4Cl) were dissolved in deionised water and made up to 1 L. The pH of the solution was adjusted to 7.4.

(B) 36.40g calcium chloride dihydrate ($\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$) was dissolved in deionised water and made up to 1 L.

(C) 22.50g magnesium sulfate heptahydrate ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$) was dissolved in

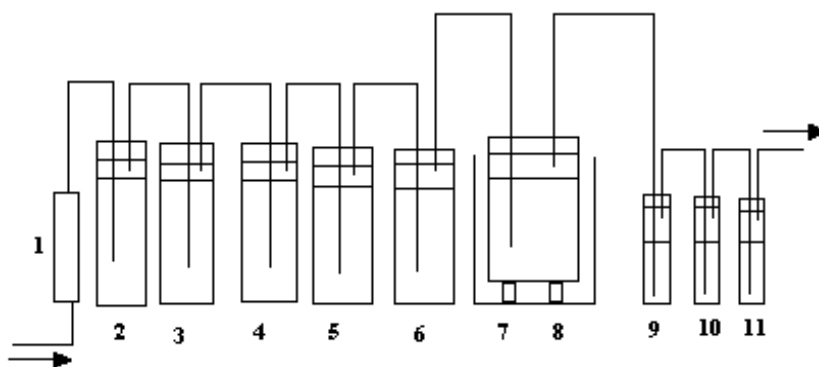
deionised water and made up to 1 L.

(D) 0.25g iron(III) chloride hexahydrate ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$) was dissolved in deionised water and made up to 1 L.

For each liter of mineral medium, 10 ml stock solution A was added to approximately 800ml of deionised water and the mixture stirred before adding, sequentially, 1ml of stock solutions B, C and D. After mixing the mixture was made up to 1 liter with deionised water. The pH was adjusted to the range of 7.4 ± 0.2 with HCl.

10.3 Equipment and apparatus

- Flasks, 2.5 litres, each fitted with an aeration tube reaching nearly to the bottom of the vessel and an outlet;
- Magnetic stirrer which type is SH23-2;
- Rotor flow meter which type is LZB-2 or LZB-3;
- TD50 Mettler Toledo automatic titrator;
- Constant water bath (22 ± 1) which type is YG-10A;
- Electron balance which type is ALB-224;
- Electro- thermostatic blast oven which type is DGG-9070A;
- pH/temperature meter which type is PB-10;
- Apparatus for carbon dioxide scrubbing;



Note: 1. Flow meter; 2. 1st CO₂ pre-absorption bottle; 3. 2nd CO₂ pre-absorption bottle; 4. 3rd CO₂ pre-absorption bottle; 5. 4th CO₂ pre-absorption bottle; 6. Scrubbing bottle; 7. Flask; 8. Water bath; 9. 1st CO₂ absorption bottle; 10. 2nd CO₂ absorption bottle; 11. 3rd CO₂ absorption bottle

10.4 Test procedures

10.4.1 Test system

Prepared test systems based on the information provided by sponsor as followed :

Table 1 Test system list

Flask No.		Contents
<u>Test Substance</u>	1	<u>Test Substance</u> (15.2 TOC mg/L.) <u>Inoculum</u> (30 mg SS/L) Nutrient medium
	2	<u>Test Substance</u> (15.2 TOC mg/L.) <u>Inoculum</u> (30 mg SS/L) Nutrient medium
<u>Inoculum Blanks</u>	3	<u>Inoculum</u> (30 mg SS/L) Nutrient medium

	4	<u>Inoculum</u> (30 mg SS/L) Nutrient medium
Process Control	5	Reference Substance (15.0 TOC mg/L.) <u>Inoculum</u> (30 mg SS/L) Nutrient medium
<u>Toxicity</u> <u>Control</u>	6	<u>Test Substance</u> (15.2 TOC mg/L.) Reference Substance (15.0 TOC mg/L.) <u>Inoculum</u> (30 mg SS/L) Nutrient medium

10.4.2 Preparation of flasks

Two treatment (containing nutrient medium, test substance and inoculums) groups, two inoculums control (containing nutrient medium and inoculums) groups, one process control (containing nutrient medium, aniline and inoculums) group, and one toxicity control (containing nutrient medium, test substance, aniline and inoculums) group were selected in the test.

1,800ml mineral medium was added to each 2.5-litre flask with an appropriate volume of the prepared activated sludge to give a concentration of suspended solids of 30 mg SS/L in the final 2 liters of inoculated mixture (see Table 1). These inoculated mixtures were aerated with CO₂-free air overnight to purge the system of carbon dioxide.

According to the formulation followed, the weight of the test substance was calculated, expressed as the theoretical amount of CO₂ (ThCO₂) that could be liberated:

$$\text{mg ThCO}_2 = \frac{(\text{percent of carbon in TS} \times 44 \times \text{mg of test substance added})}{12}$$

TS: Test substance.

The theoretical yield of CO₂ for the Process Control (PC) was also calculated. A weight of 39.0 mg aniline was used, giving a ThCO₂ of 110.0 mg.

10.4.3 Preparation of CO₂ pre-absorption bottles

Five 500 ml absorption flasks were used as CO₂ pre-absorption and scrubbing bottles. The 1st, 2nd and 3rd bottles contained 350 mL 10 mol/L NaOH, the 4th contained 350 mL 0.05 mol/L Ba(OH)₂, and the 5th bottle contained distilled water.

10.4.4 CO₂ absorption bottle

Three 250 mL absorption flasks were selected containing 100 mL 0.0125 mol/L Ba(OH)₂ to absorb CO₂ produced from biodegradation.

10.4.5 Bioassay conditions

Brown flasks were used to avoid the direct light and kept at 22±1 °C in a water bath. The airflow rate was 30 mL/min-100 mL/min.

10.4.6 Determination of CO₂

Determination of CO₂ was carried out at 2, 4, 6, 8, 10, 12, 14, 18, 22, 26 and 29 days after the beginning of the test (see Table 2). On the days of CO₂ measurement, the barium hydroxide absorber closest to the test vessel was disconnected and the hydroxide solution was titrated with 0.0504 M HCl using phenolphthalein as the indicator. The remaining absorbers were moved one place closer to the test vessel and a new absorber containing fresh barium hydroxide was placed at the far end of the series. The samples were titrated as needed and CO₂ contents were determined.

On the 28th day, samples were withdrawn and the pH was measured (see Table 3). Then 1 ml of concentrated hydrochloric acid was added to each test vessel and they were aerated overnight to drive off dissolved carbon dioxide present in the test suspensions. On day 29 the last analyses of evolved carbon dioxide were made.

10.5 Treatment of results

The weight of CO₂ produced (mg) was calculated by:

$$\text{CO}_2 \text{ production (mg)} = 0.0504 \times [50.0 - \text{HCl titrated volume (ml)}] \times 44/2$$

The percentage biodegradation was calculated by:

$$\% \text{ degradation} = \frac{\text{mg CO}_2 \text{ produced (mean TS or PC - mean IC)}}{\text{ThCO}_2 \text{ mg for test substances added}} \times 100$$

Note : TS means test substance;

PC means process control;

IC means inoculum blank.

The course of each degradation was presented graphically; the graph indicated that the test material had ready biodegradability judged by the 10-day window appropriate for classification. The degradation rate at the end of the test was 8.36%.

10.6 Validity of tests

The IC content of the test substance suspension in the mineral medium was 290µg/mL at the beginning of the test. It did be less than 5% of the TC.

The mean total CO₂ evolution in the inoculum blank was 24.64mg/L at the end of the test, therefore, it did not exceed 40 mg/L medium as required in the guidelines.

In the process controls, 76.57% degradation was achieved in 14 days, within the period required by the guidelines.

In the toxicity controls, 46.83% degradation was achieved in 14 days. It exceeded 25%, that could be considered that the test substance with the test concentration did not inhibit the activated sludge.

At the end of the test, the biggest difference of degradations between parallels is 0.17%. It did not exceed 20% as required in the guidelines.

11、 Results and discussion

11.1 Presentation of the results

██████████ as added to the culture medium at a level equating to 15.2 mg TOC/L. The accumulated percentage biodegradation of the test substance in activated sludge after 2, 4, 6, 8, 10, 12, 14, 18, 22, 26 and 29 days were 1.63%, 3.56%, 5.09%, 6.00%, 7.36%, 7.41%, 7.48%, 7.60%, 7.95%, 8.24% and 8.36%, respectively. Therefore, the accumulated percentage biodegradation rate of the test substance was 8.36% in 29-day.

The accumulated percentage biodegradation of the reference substance (aniline) in activated sludge after 2, 4, 6, 8, 10, 12, 14, 18, 22, 26 and 29 days were 2.09%,

22.31%, 52.67%, 61.15%, 67.84%, 73.66%, 76.57%, 79.64%, 81.28%, 83.03% and 83.19%, respectively.

11.2 Conclusion

██████████ was not concluded to be easily (readily) biodegradable in this test condition.

12、Deviation from study plan

/

13、Archives

The following will be retained in the testing centre archives room for three inspection cycles after the testing report is submitted to sponsor. : (1) raw data and final report, (2) records of test substance and the reference item, (3) study plan.

The test item will be retained in the testing centre sample room for six months.

The sponsor can consult with testing center if the longer retain time is needed.

Address of test facility sample storage room:

No.4 Building Room 614

No. 345 East Yunling Road, Shanghai

Shanghai Research Institute of Chemical Industry

The contact way of Archivist:

Tel : +86 021 52815377-1701

Fax : +86 021 52815377-1713

E-mail: kulkkk@hotmail.com

14、Attached tables

Table 2. CO₂ Production and Reference During the 29 Days Testing

Day (d)	CO ₂ Produced (mg)						Degradation% = $100 \times \frac{(\text{CO}_2 \text{ (mg) Mean (TS(PC)} - \text{Mean IC)})}{\text{ThCO}_2 \text{ (mg)}}$			Cumulative % Degradation		
	Inoculum Control		Test Substance		Process Control	Toxicity Control.						
	IC-1	IC-2	TS-1	TS-2	PC	TC	TS	PC	TC	TS	PC	TC
2	2.89	2.99	4.95	4.56	5.23	6.95	1.63	2.09	1.81	1.63	2.09	1.81

4	4.47	3.57	5.88	6.46	26.27	30.47	1.93	20.22	11.95	3.56	22.31	13.76
6	7.04	6.50	8.76	8.17	40.17	40.35	1.52	30.36	15.17	5.09	52.67	28.93
8	2.41	2.69	3.34	3.79	11.88	17.60	0.91	8.48	6.80	6.00	61.15	35.72
10	2.79	2.69	4.55	3.97	10.11	14.76	1.37	6.70	5.43	7.36	67.84	41.15
12	5.62	4.50	5.09	5.13	11.46	12.45	0.05	5.82	3.34	7.41	73.66	44.49
14	4.43	3.43	3.94	4.08	7.13	9.10	0.07	2.91	2.34	7.48	76.57	46.83
18	4.17	3.64	3.93	4.15	7.28	8.13	0.12	3.06	1.91	7.60	79.64	48.73
22	4.68	3.54	4.35	4.66	5.92	7.82	0.35	1.65	1.68	7.95	81.28	50.41
26	4.83	4.93	5.07	5.32	6.80	6.66	0.29	1.75	0.81	8.24	83.03	51.22
29-1	6.33	4.96	5.90	5.68	5.82	7.84	0.13	0.16	0.99	8.36	83.19	52.21
29-2	2.81	2.64	2.71	2.67	2.72	3.07	0.00	0.00	0.16	8.36	83.19	52.36
29-3	1.64	2.27	1.78	1.94	1.95	2.17	0.00	0.00	0.10	8.36	83.19	52.46

Note: IC means Inoculum Control; TS means Test Substance; PC means Process Control and TC means Toxicity Control.

If CO₂ production (mg) (TS-IC) <0, it is considered that biodegradation is temporarily prohibited, and values of the cumulative CO₂ production are constant.

29-1 means the first CO₂ absorption bottle on the 29th day,

29-2 means the second CO₂ absorption bottle on the 29th day,

29-3 means the third CO₂ absorption bottle on the 29th day.

Table 3. pH Measured at the Beginning and the End of the Test

Treatment	pH	
	At the beginning (0d)	At the end (28d)
IC-1	7.41	7.16
IC-2	7.41	7.16
TS-1	7.40	7.16
TS-2	7.40	7.16
PC	7.40	7.10
TC	7.40	7.11

Note: IC means Inoculum Control; TS means Test Substance; PC means Process Control and TC means Toxicity Control.

Graph 1 Biodegradation Curve of Revonox 501

